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NEGATIVE DOMINANT MUTATIONS OF THE *uidR* GENE IN *ESCHERICHIA COLI*: GENETIC PROOF FOR A COOPERATIVE REGULATION OF *uidA* EXPRESSION

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ABSTRACT

The *uidA* gene is the first gene involved in the hexuronide-hexuronate pathway in *Escherichia coli* K-12 and is under the dual control of the *uidR* and *uxuR* encoded repressors. Point mutations affecting the *uidR* regulatory gene were sought to investigate the regulation of *uidA*. When the *uidR* mutant allele was on a multicopy plasmid and the wild-type allele was on the chromosome, some of the mutant phenotypes were dominant to the wild-type phenotype, indicating that the active form of the UidR repressor is multimeric. We have demonstrated that expression of the mutant phenotype is dependent on gene dosage. The dominance of the *uidR* allele was also sensitive to the presence of the wild-type *uxuR* allele in the cell. This behavior probably results from UidR-UxuR repressor interactions. A mechanism is proposed: we suggest that the UidR and UxuR repressors interact after their binding to the operator site of *uidA*; the binding of one regulatory molecule may facilitate the binding of the other one in a cooperative process.

THE *uidA* gene, located at min 36 (BACHMANN 1983), codes for β-glucuronidase, which is the first enzyme of the hexuronide-hexuronate pathway in *Escherichia coli* K-12 (ASHWELL 1962; STOEBER 1961). Expression of *uidA* is principally regulated by the *uidR* encoded repressor and secondarily by the UxuR repressor (NOVEL and NOVEL 1976). This latter repressor also exerts a control on the *uxuAB* operon (ROBERT-BAUDOUY, PORTALIER and STOEBER 1981). The mechanism of this dual control is unknown. Glucuronate is able to relieve the repression produced by the UxuR repressor on *uidA* expression, whereas methyl-β-glucuronide antagonizes the repression exerted by UidR. The three *uidR*, *uxuR* and *uidA* genes have been cloned into the plasmid pBR322 or derivatives (RITZENTHALER, MATA-GILSINGER and STOEBER 1980; BLANCO, RITZENTHALER and MATA-GILSINGER 1982). In a previous report (RITZENTHALER, BLANCO and MATA-GILSINGER 1983), we showed that UxuR and UidR repressors are partially interchangeable for the control of *uidA*

TABLE 1

Bacterial strains and plasmids used in this study

Designation	Relevant genotype	Source
Bacterial strains		
MC4100	*ΔlacU169 araD139 rpsL*	CASADABAN (1976)
2510	As MC4100 but *recA1*	This laboratory
2849	As 2510 but *uidR*	This laboratory
3119	As 2510 but *ΔuxuR101 uxu-B100*::Mud(Ap^r *lac*)	RITZENTHALER and MATA-GILSINGER (1983)
1830	*uidA manA arg lac gal mtl xyl*	This laboratory
2523	*uidR recA1 rpsL fadD88 gal*	This laboratory
PR1	*uxuR14 uxuA1 uxaB3 argH thr leu recA1 rpsL*	This laboratory
Plasmids		
pBR322	*bla*$^+$ *tet*$^+$	BOLIVAR *et al.* (1977)
pACYC184	*tet*$^+$ *cat*$^+$	CHANG and COHEN (1978)
pCB101	*cat*$^+$ *uidR*$^+$	Derived from pACYC184
pCB8	*tet*$^+$ *uidR*$^+$	Derived from pBR322
pCB8-91 to 97[a]	As pCB8 but *uidR*$^-$	This study
pCBGR36	*cat*$^+$ *uidR-lacZ*	BLANCO, MATA-GILSINGER and RITZENTHALER

[a] Plasmids pCB8-91 to 97 are derivatives from pCB8, with point mutation(s) in the cloned *uidR* gene.

because addition of multicopy plasmids bearing *uidR*$^+$ to *uxuR* deleted mutant strains partially suppresses the derepression of *uidA*; nevertheless, the complete repression of *uidA* requires the presence of the two regulatory molecules.

Here, we try to investigate the structure of the UidR repressor by a genetic approach. Dominant negative *uidR* mutants were isolated by *in vitro* mutagenesis of the cloned regulatory gene. The mutants are described and implications for the structure of the UidR repressor and for the UxuR-UidR interactions are discussed.

MATERIALS AND METHODS

Bacterial strains and plasmids: The bacterial strains were *E. coli* K-12 derivatives. Bacterial strains and plasmids used in this investigation are listed in Table 1.

Culture media: Media for growth were identical to those described by MILLER (1972). Synthetic medium was either M63 (SISTROM 1958) or M9 (MILLER 1972) and contained either glycerol (5 g/liter) or glucuronate (2.5 g/liter). When needed, ampicillin and chloramphenicol were used at final concentrations of 25 μg/ml, and tetracycline was added at 15 μg/ml.

Enzyme assays: β-glucuronidase and mannonate oxidoreductase were assayed according to previously published methods (NOVEL and NOVEL 1976; ROBERT-BAUDOUY, PORTALIER and STOEBER 1974). β-galactosidase was assayed by the method of MILLER (1972) in exponentially growing cells.

Isolation and analysis of plasmid DNA: Procedures for isolation of plasmid DNA (BIRNBOIM and DOLY 1979; GUERRY, LE BLANC and FALKOW 1973), purification of the DNA by dye-buoyant density centrifugation in CsCl gradients (RADLOFF, BAUER and

VINOGRAD 1967) and transformation of *E. coli* with plasmid DNA (MANDEL and HIGA 1970) have already been described.

Isolation of mutations in the cloned *uidA* or *uidR* gene: Mutagenesis of plasmid DNA harboring the *uidR* gene was carried out in a modified version of the TESSMAN (1968) method for phage: the plasmid DNA was incubated at 70° in a solution of 0.8 M hydroxylamine, pH 6; 0.1 M phosphate buffer, pH 6; and EDTA 10^{-3} M for 200 min. The mutagenized DNA was dialyzed against 75 mM $CaCl_2$, precipitated in ethanol and used to transform various strains. The plasmid used in this mutagenesis was pCB8. The *uidR* gene has been cloned into the ampicillin resistance gene of pBR322 yielding pCB8 (BLANCO, RITZENTHALER and MATA-GILSINGER 1982).

RESULTS

Isolation and characterization of mutations affecting the *uidR* regulatory gene: Plasmid pCB8 (BLANCO, RITZENTHALER and MATA-GILSINGER 1982) carrying a functional *uidR* gene was mutagenized as described in MATERIALS AND METHODS. Strain 2523 (*uidR*) was used to select for *uidR* mutant plasmids. After transformation of this strain by the mutagenized plasmid, 62 (2%) of 3,000 transformants retained constitutive expression of *uidA*, whereas this constitutive expression was completely abolished in the presence of wild-type pCB8. These data indicated that the 62 mutant plasmids derived from pCB8 carried a defective *uidR* gene. To find out whether *uidR* missense mutations are negatively dominant, the mutant plasmids were introduced by transformation into various strains, and the β-glucuronidase specific activity was measured in these strains. Other negative dominant mutations (*uidR*$^{-d}$) were directly selected using strain 2510, which bears a wild-type *uidR* gene. This strain was transformed by the mutagenized pCB8 plasmid, and the transformant clones were screened for the presence of β-glucuronidase activity in the absence of inducer. Of 2,000 clones tested, 20 showed constitutive synthesis of β-glucuronidase. Expression of seven representative mutant plasmids selected in strain 2523 or 2510 is shown in Table 2. In 2523 (*uidR*), all the mutant plasmids were not able to suppress completely the constitutive expression of *uidA*, contrary to wild-type pCB8. Various levels of β-glucuronidase synthesis were obtained, from 2% (pCB8-95) to 100% (pCB8-96) of the fully derepressed level. In strain 2510 (*uidR*$^{+}$), addition of the mutant plasmids caused a weak derepression of the β-glucuronidase synthesis (3.5–11% of the fully induced level), except in the case of pCB8-93, which represents the negative recessive mutation-type of *uidR*. In the presence of the mutant plasmids, the levels of constitutive β-glucuronidase synthesis in the wild-type strain and in the *uidR* mutant strain were not correlated. These results show that the missense mutations of the plasmids are negatively dominant over the wild-type function, except for the *uidR93* mutation carried by pCB8-93; this suggests an oligomeric nature for the UidR repressor.

An alternative possibility can be proposed: the mutant *uidR* alleles produced altered repressors that were defective in repressing at the *uidA* operator, but were still effective in repressing the expression of *uidR* because *uidR* was shown to negatively regulate its own synthesis (BLANCO, MATA-GILSINGER and RITZENTHALER 1985). To examine this hypothesis, a previously constructed *uidR*-

TABLE 2

***uidA* gene expression in various strains transformed by plasmids bearing *$uidR^{-d}$* alleles**

Plasmid	*uidR* allele on plasmid	Inducer[a] (5 mM)	β-Glucuronidase specific activity in strains			
			2523 (*$uxuR^+$ $uidR^-$*)	2510 (*$uxuR^+$ $uidR^+$*)	PR1 (*$uxuR^-$ $uidR^+$*)	3119 (*Δ$uxuR$ $uidR^+$*)
pBR322	None	−	5350 (100)[b]	1 (<0.1)	34 (<1)	38 (<1)
pBR322	None	+		3000 (100)	4010 (100)	4700 (100)
pCB8	*$uidR^+$*	−	3 (<0.1)	1 (<0.1)	3 (0.1)	20 (<1)
pCB8-91	*uidR91*	−	3370 (63)	106 (3.5)	523 (13)	700 (15)
		+		2950 (98)		
pCB8-92	*uidR92*	−	4260 (80)	210 (7)	1290 (32)	1730 (37)
pCB8-94	*uidR94*	−	830 (15)	153 (5)	1420 (35)	2480 (53)
		+		2890 (96)		
pCB8-95	*uidR95*	−	98 (2)	195 (6)	1700 (42)	1990 (42)
pCB8-96	*uidR96*	−	5480 (102)	330 (11)	3610 (90)	5120 (109)
pCB8-97	*uidR97*	−	1140 (21)	300 (10)	2110 (52)	3000 (64)
pCB8-93	*uidR93*	−	3370 (63)	4 (<1)	47 (1)	48 (1)

[a] The inducer was methyl-β-glucuronide.

[b] Numbers within parentheses represent the percentage of the value determined in the presence of plasmid pBR322 under noninduced conditions (first column) or induced conditions (other columns). The specific activities used as reference are different for each strain.

TABLE 3

Effect of *$uidR^{-d}$* alleles on *uidR-lacZ* gene expression in strains 2510 and 2849 containing *uidR-lacZ* fusion plasmid pCBGR36

Plasmid *in trans*	β-Galactosidase activity[a] (units/mg) in strains	
	2510 (Δ*lac*)	2849 (*uidR* Δ*lac*)
pBR322	2500	2500
pCB8	1500	1500
pCB8-91	2300	2300
pCB8-92	2500	2500
pCB8-94	2300	2300
pCB8-95	2200	2200
pCB8-97	2200	2300
pCB8-93	2300	2300

[a] One unit of β-galactosidase activity is the amount of enzyme that hydrolyzes 1 nmol of *o*-nitrophenylgalactoside per min.

lac fusion carried by plasmid pCBG36 was used (BLANCO, MATA-GILSINGER and RITZENTHALER 1985). A significant decrease in β-galactosidase synthesis was observed in strain 2510 carrying pCBG36 when the wild-type *uidR* gene of pCB8 was added. In contrast, the *uidR* mutated plasmids failed to repress the expression of the hybrid *lacZ* gene (Table 3). The *$uidR^{-d}$* mutants did not enhance the synthesis of the *uidR-lacZ* encoded protein contrary to that of β-glucuronidase, because the UidR repressor only weakly repressed its own synthesis (about 35%). These results exclude the possibility that the *uidA* de-

TABLE 4

Effect of *uidR*$^{-d}$ alleles on *uxuB* expression in strains 3119 (*uxuB-lac* Δ*uxuR uidR*$^{+}$) and 2510 (*uidR*$^{+}$ *uxuR*$^{+}$)

Plasmid	Specific activities	
	β-Galactosidase (*uxuB-lac*) units/mg in strain 3119	Mannonate oxidoreductase[a] (*uxuB*) milliunits/mg in strain 2510
pBR322	2040(0)[b]	40
pCB8	1200(41)	20
pCB8-91	1780(13)	30
pCB8-92	1270(38)	40
pCB8-94	1880(8)	40
pCB8-95	1990(3)	45
pCB8-96	2050(0)	40
pCB8-97	1790(12)	30
pCB8-93	1680(17)	40

[a] One unit of mannonate-oxidoreductase activity is the amount of enzyme that converts 1 μmol of substrate per min.

[b] The numbers within parentheses represent the percentage of the repression exerted by pCB8 and derivatives.

creased repression observed in the presence of the *uidR* mutated plasmids is due to a decrease in the UidR repressor synthesis and support the hypothesis of negative dominant mutations.

The presence of a chromosomal *uxuR* mutation (missense mutation in strain PR1 or deletion in strain 3119) enhanced the derepression effect of the mutant plasmids on β-glucuronidase synthesis (from four to eightfold of that obtained in the wild-type strain 2510) (Table 2). When methyl-β-glucuronide, which inactivates the wild-type UidR repressor, was used as inducer, the fully induced β-glucuronidase activity was not affected by the addition of the mutated plasmid (see Table 2a, strain 2510 containing pCB8-94 for example). The UidR^{-d} repressor mutants seem to have defects in the DNA binding site, but not in the inducer binding site.

Effect of the *uidR*$^{-d}$ alleles on *uxuAB* expression: It has already been observed that introduction of plasmid pCB8 into strain PR1 (*uxuR*) caused a 35% decrease in the derepressed synthesis of the *uxu* enzymes due to the overproduction of the UidR repressor (RITZENTHALER, BLANCO and MATA-GILSINGER 1983). We attempted to examine whether the *uidR*$^{-d}$ plasmids derived from pCB8 have a similar effect on the constitutive expression of the *uxu* operon. Therefore, the *uxu* enzyme synthesis was measured in strain 3119 carrying the various mutant plasmids. In this strain, the *uxuR* gene is deleted and *lacZ* is fused to *uxuB*, so that β-galactosidase synthesis reflects *uxuB* gene expression. In all cases, the repression exerted by the *uidR*$^{-d}$ alleles was similar or lower than that exerted by the wild-type allele (Table 4). This lower repression probably results from the partial inactivation of the UidR repressor due to mutation.

The *uxuAB* expression was also examined in strain 2510 (*uxuR*$^{+}$, *uidR*$^{+}$).

TABLE 5

Effect of the *uidR*$^+$ copy number on the expression of the *uidR*$^{-d}$ phenotype in strain 2510

Plasmid	Plasmid *in trans*	β-Glucuronidase specific activity (milli-units/mg)	Residual activity (%)[a]
pBR322	None	1	
pCB8-94	None	153	100
pCB8-94	pACYC184 (control)	100	65
pCB8-94	pCB101 (*uidR*$^+$)	16	10
pCB8-95	None	195	100
pCB8-95	pACYC184	116	60
pCB8-95	pCB101	20	10

[a] The residual activity is the β-glucuronidase activity that remains in strain 2510 harboring *uidR*$^{-d}$ plasmids after addition of another plasmid *in trans.*

Contrary to β-glucuronidase synthesis, which was increased by addition of the *uidR*$^{-d}$ plasmid (Table 2), *uxu* enzyme synthesis was unchanged when the mutant plasmids were introduced into strain 2510 (Table 4). The *uidR*$^{-d}$ mutations do not affect the control of the *uxuAB* operon.

Sensitivity of the *uidR*$^-$ mutation expression to the gene dosage of *uidR*$^+$: In most cases where negative dominance was observed, the *uidR* mutation expression was strong because there was a large excess of the *uidR* missense product (encoded by the multicopy plasmid) over the wild-type repressor (encoded by the chromosome). In order to demonstrate that expression of the mutant phenotype is sensitive to the amount of functional UidR repressor in the cell, the relative copy number of *uidR*$^+$ was increased. This was carried out by introducing into strain 2510 one of the *uidR*$^{-d}$ plasmids and the compatible plasmid pCB101 carrying a functional *uidR* gene. In these conditions, the cell contained approximately equal number of copies of the *uidR*$^{-d}$ and *uidR*$^+$ alleles. The constitutive expression of the *uidA* gene was strongly reduced by the simultaneous presence of the two plasmids (Table 5). Therefore, expression of the mutant phenotype is dependent on gene dosage and the magnification of partial dominance results from the overproduction of the missense product.

DISCUSSION

In this work, we describe the isolation of *uidR* negative dominant mutations. The existence of this type of mutations has been reported for various multisubunit proteins, such as *lac* operon repressor (DAVIES and JACOB 1968; MÜLLER-HILL, CRAPO and GILBERT 1968), *phoR* gene product (PRATT and GALLANT 1972) or *glpT*-encoded permease (LARSON, SCHUMACHER and BOOS 1982). The dominance of the mutant phenotype over the wild-type allele has been explained by the formation of mixed oligomers between the wild-type and mutant subunits of the protein and has been termed anticomplementation or negative complementation. Anticomplementation was demonstrated *in vitro*

for Neurospora glutamate dehydrogenase (SUNDARAM and FINCHMAN 1967), *lac* repressor (MÜLLER-HILL, CRAPO and GILBERT 1968) and *capR* protein (CHARETTE *et al.* 1982). In these cases, mixtures of purified wild-type and mutant proteins had less activity than the wild-type protein treated similarly.

The mixed $uidR^{+}$-$uidR^{-d}$ oligomers brought about a constitutive expression of *uidA*, but are still sensitive to inducer; therefore, the *uidR* negative dominant mutants seem to have defects in the operator binding site, but no defect in the inducer binding site. We assume that the mutations do not affect the sites of self-association; the wild-type and mutant subunits presumably compete at random for positions in the oligomer.

We have demonstrated that expression of the *uidR* mutant phenotype is dependent on gene dosage. This sensitivity to gene dosage explains why $uidR^{-d}$ mutant could easily be isolated only when the mutant allele was carried on a multicopy plasmid.

The $uidR^{-d}$ mutations do not modify *uxuAB* operon expression in a wild-type strain, contrary to the behavior of $uxuR^{-d}$ mutations (RITZENTHALER, BLANCO and MATA-GILSINGER 1985). The original effect that we have found is the sensitivity of *uidR* anticomplementation to the presence of the wild-type *uxuR* allele in the cell: the *uidA* gene derepression caused by addition of $uidR^{-d}$ alleles is strong in the absence of a functional *uxuR* gene and is significantly reduced when an active UxuR repressor is synthesized. This behavior probably results from UidR-UxuR repressor interactions. Such interactions were previously predicted to explain the partial interchangeability of UxuR and UidR repressors for the control of *uidA* (RITZENTHALER, BLANCO and MATA-GILSINGER 1983): full repression of *uidA* expression is achieved only in the simultaneous presence of both UxuR and uidR repressors, even when UidR or UxuR is overproduced in the cell. To explain the repressor interchangeability and the behavior of the $uidR^{-d}$ mutations, two hypotheses can be put forward:

1. UidR and UxuR repressors form a complex that is the true repressor. This complex may consist of the two entire repressors or may consist of association of UidR and UxuR monomers, because UxuR is also a multimeric molecule (RITZENTHALER, BLANCO and MATA-GILSINGER 1985).

2. The UidR-UxuR interactions occur after the binding of the repressors to DNA. Each repressor can bind to DNA separately, but the binding of one regulatory molecule facilitates the binding of the second in a cooperative process.

The first hypothesis may be ruled out because the formation of the UidR-UxuR complex in cells that overproduce UidR repressor would titrate out UxuR repressor, resulting in the derepression of *uxuAB* operon; this is not the case. Moreover, an excess of UidR repressor causes full *uidA* repression in some *uxuR* missense mutants, such as PR1, suggesting that even the altered UxuR repressor helps UidR to bind to the *uidA* operator site or vice versa.

Such mechanism of interactions between two DNA binding molecules to maximize their action has been described by various authors to explain positive control of DNA transcription. For example, the λ repressor stimulates the transcription of its own gene by binding to the λ operator and contacting the

RNA polymerase that binds to the adjacent promoter (HOCHSCHILD, IRWIN and PTASHNE 1983). HAWLEY and MCCLURE (1983) describe a λ repressor mutant, the behavior of which suggests that direct repressor-RNA polymerase interactions are important in the P_{RM} activation mechanism. A cooperative model for the λ DNA-bound repressor interactions is proposed by JOHNSON, MEYER and PTASHNE (1979). Contrary to that found for the *cro* protein, the λ repressor binds cooperatively to the three O_R operators. The repressor interactions are probably mediated by protein-protein contacts between adjacent repressor dimers.

The ability of the UxuR and UidR repressors to interact with each other seems to need the binding of one of the two regulatory molecules to the operator site. We suppose that the cooperative mechanism is dependent on this first step. We can imagine a process similar to the activation of the *recA* protein proteolytic activity in which the activation requires the formation of a ternary complex between the *recA* protein, ATP and single-strand DNA (CRAIG and ROBERTS 1980).

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